Attorney's Docket No.: 08952-008001 / UMA 00-19

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Elizabeth S. Stuart et al.
Serial No. : 09/827,490
Filed : April 6, 2001
Title : CHLAMYDIAL GLYCOLIPID VACCINES

Art Unit : 1645
Examiner : Vanessa L. Ford

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

I, Lloyd Semprevivo, declare that:

1. I am a co-inventor of the subject matter claimed in the patent application captioned above ("the present application").

2. I understand that claim 15 of the present application has been rejected by the U.S. Patent & Trademark Office Examiner in a final Office Action dated May 16, 2003, as allegedly anticipated by Stuart et al. (*Immunology*, 68:469-473 (1989)). I have reviewed this reference. According to the Office Action, Stuart discloses purified chlamydial glycolipids that are free of other components as determined by sodium dodecylsulfate gel electrophoresis and silver staining. Further, the Office Action indicates that applicants should provide a side-by-side comparison to show that the preparation recited in claim 15 is different from those disclosed in Stuart.

3. The present declaration provides the side-by-side comparison requested in the Office Action. Specifically, Figs. 1 to 3, below, show the significant progression in methodology for

isolating GLXA from infected culture supernatants and provide silver stained gels of the preparations. Note that Fig. 1, below, is a duplicate of Stuart's Fig. 2.

4. Fig. 1 is a picture of a silver stained 8-25% SDS-PAGE PhastGel™ of GLXA purified from the supernatant of cultures infected with different serovars of chlamydia [lanes a-d]. Molecular weight standards are included [lane e]. This figure (reproduced from Stuart) illustrates the purity of preparations obtained using early methodology involving Octyl-Sepharose hydrophobic chromatography to initially isolate antigen, followed by re-isolation of 'shifted' GLXA using potassium thiocyanate (KSCN) and Octyl-Sepharose. Note that when the polyclonal antibody is used, other chlamydial components are isolated from the culture media, along with GLXA. As a result, lanes a to d show many bands, indicating that a substantial amount of contaminating materials is present along with the GLXA.

5. Figs. 2 and 3 are pictures of a silver stained gel and a Western blot, respectively, illustrating results obtained using the protocol described in the present application. In the protocol, supernatants from infected cultures are ultracentrifuged and subjected to DNAse, RNAse, and mAb1 affinity column treatment, as described in the specification, e.g., at page 2, lines 10 to 20. Clearly, this protocol results in a GLXA composition that is significantly better defined than those obtained using the previous protocol. Samples were analyzed by electrophoresis using an 8-16% Tris-Glycine pre-cast Novex™ gel. Aliquots were electrophoresed and then transferred to polyvinylidene fluoride (PVDF) membrane and immuno-probed using monoclonal antibody (89MS30) (see Fig. 3). In Fig. 2 and Fig. 3, GLXA appears as the bands having molecular weights of about 62 kDa and 32 kDa (the 32 kDa band is likely a fragment or subunit of the 62 kDa band). This product can subsequently be used to generate the isolated oligosaccharides described in the present application.

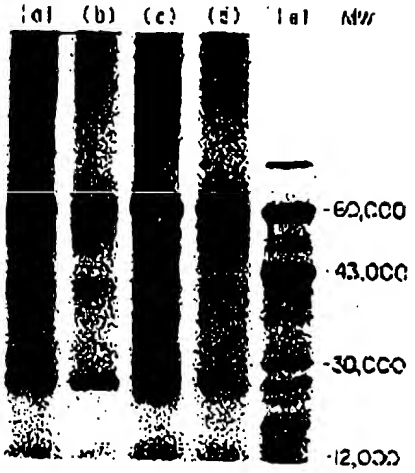

Fig. 1

Fig. 2

Fig. 3

I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 13 Nov 03

Lloyd H. Semprevivo, Ph.D.

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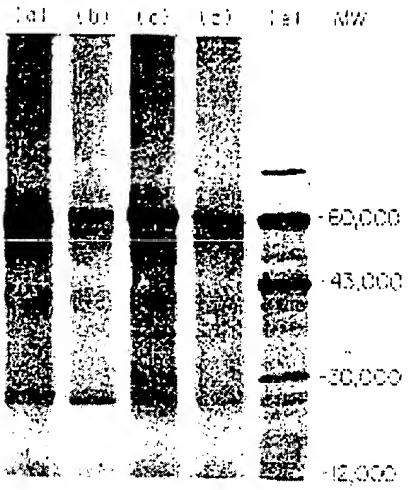

Fig. 1

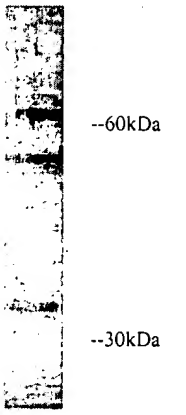

Fig. 2

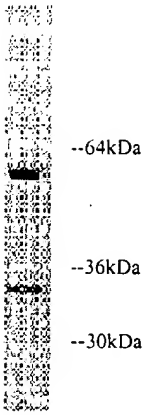

Fig. 3

I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date:_____

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